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Cochlodinium fulvescens sp. nov. (Gymnodiniales, Dinophyceae), a new chain-forming unarmored dinoflagellate from Asian coasts

Mitsunori Iwataki,[1]* **Hisae Kawami**[2] **and Kazumi Matsuoka**[1]

[1]Institute for East China Sea Research, Nagasaki University, Nagasaki 852-8521, Japan, and [2]Graduate School of Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan

SUMMARY

Cellular morphology and the phylogenetic position of a new unarmored photosynthetic dinoflagellate *Cochlodinium fulvescens* Iwataki, Kawami et Matsuoka sp. nov. were examined by light microscopy and molecular phylogenetic analyses based on partial large subunit ribosomal DNA (LSU rDNA) and small subunit ribosomal DNA (SSU rDNA) sequences. The cells of *C. fulvescens* closely resemble *C. polykrikoides*, one of the most harmful red tide forming dinoflagellates, due to it possessing a cingulum encircling the cell approximately twice, a spherical nucleus positioned in the anterior part of the cell and an eyespot-like orange pigmented body located in the dorsal side of the epicone, as well as formation of cell-chains. However, this species is clearly distinguished from *C. polykrikoides* based on several morphological characteristics, namely, cell size, shape of chloroplasts and the position of narrow sulcus situated in the cell surface. The sulcus of *C. fulvescens* is located at the intermediate position of the cingulum in the dorsal side, whereas that of *C. polykrikoides* is situated immediately beneath the cingulum. LSU rDNA phylogenies indicated that *C. fulvescens* is clearly distinct from, but closely related to *C. polykrikoides* among dinoflagellates.

Key words: *Cochlodinium*, *Cochlodinium fulvescens* sp. nov., *Cochlodinium polykrikoides*, dinoflagellate, Dinophyceae, Gymnodiniales, large subunit ribosomal DNA.

INTRODUCTION

The unarmored dinoflagellate genus *Cochlodinium* was established by Schütt in 1896, including the species with the cingulum encircling the cell more than one and a half times (Schütt 1896; Kofoid & Swezy 1921). Since the proposal of the genus in 1896, more than 40 species have been assigned to this genus based on the characteristics of the cingulum. Most of these *Cochlodinium* species are heterotrophic, and have rarely been observed since their original descriptions. On the other hand, a photosynthetic species of the genus, *Cochlodinium polykrikoides* Margalef has been reported intensively as a red tide forming species in connection with serious fish mass mortalities, from coastal waters of the tropical-temperate zones of the Pacific, Atlantic and Indian Oceans (Lu & Hodgkiss 1999; Gárate-Lizárraga *et al*. 2000; Morales-Blake *et al*. 2001; Whyte *et al*. 2001; Relox & Bajarias 2003; Bhat & Matondkar 2004; Matsuoka & Iwataki 2004; Nuzzi 2004; Vargas-Montero *et al*. 2004; Azanza & Baula 2005). On account of the noxious effect for culture fishes, morphology, ecophysiology and occurrences of red tides of *C. polykrikoides* have been well investigated (e.g. Yuki & Yoshimatsu 1989; Park *et al*. 2001; Kim *et al*. 2002, 2004a,b; Jeong *et al*. 2004; Yamatogi *et al*. 2005a,b). Among these reported occurrences, several morphotypes resembling *C. polykrikoides* have been observed and described as unidentified *Cochlodinium* sp. (e.g. Yuki & Yoshimatsu 1989; Whyte *et al*. 2001). However, taxonomic significance of their morphological differences and phylogenetic relationships to *C. polykrikoides* remain obscure.

From the coasts of Indonesia and Japan, a chain-forming photosynthetic *Cochlodinium* species closely resembling *C. polykrikoides* but also having distinctive characteristics was isolated. In the present study, we propose a new unarmored dinoflagellate *Cochlodinium fulvescens* Iwataki, Kawami et Matsuoka sp. nov. assigned to the Gymnodiniales, Dinophyceae, based on morphological and molecular phylogenetic evidence.

MATERIALS AND METHODS

Cultures

Cells of *Cochlodinium fulvescens* examined the morphology, and large subunit ribosomal DNA (LSU rDNA)

*To whom correspondence should be addressed.
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(28S ribosomal RNA gene) and small subunit rDNA (18S ribosomal RNA gene) sequences were isolated from plankton samples collected off Tachibana Bay, Nagasaki (32°32′N, 129°42′E) in October 2004. A unialgal culture was established and maintained using Erd-Schreiber modified (ESM) medium (Watanabe *et al*. 2000) under conditions of 20°C with an irradiance of 100 μmol photons m^{-2} s^{-1}. The other specimen of *C. fulvescens* was collected from Hurun Bay, Lampung, Indonesia (05°31′S, 105°15′E) in December 2003. A culture of *C. polykrikoides* used for the morphological and phylogenetic analysis was isolated from seawater collected at Nama Bay, Kamigoto Island, Nagasaki, Japan (33°01′N, 129°04′E) in August 2002. LSU rDNA sequences of three other *C. polykrikoides* cultures, which were collected from Inokushi Bay, Oita (32°48′N, 131°53′E) in May 2004, from Tachibana Bay, Nagasaki (32°40′N, 130°08′E) in July 2002, and from Usuka Bay, Nagasaki (33°23′N, 129°32′E) in October 2003, were also included to the phylogenetic analyses.

Microscopy

Cells of *Cochlodinium* were observed under a light microscope (Olympus BX51, Tokyo, Japan) equipped with an epifluorescence device, and photos were taken by a digital camera model Olympus DP50. Shape and position of chloroplasts were observed by its autofluorescence under a blue excitation.

Sequence analyses

Partial LSU rDNA (D1-D6) sequences were determined by direct sequencing with suitable primer sets by the following procedure. Total DNA were extracted from the *Cochlodinium* cultures using a SepaGene kit (Sanko-Junyaku, Tokyo, Japan) according to the manufacturer's instructions. DNA amplification of partial LSU rDNA sequence (approximately 1500 bp) was carried out using the purified total DNA as a template, in a 20-μL reaction volume with the reagents according to the manufacturer's recommendation of KOD-Plus DNA polymerase (Toyobo, Osaka, Japan). Terminal primers for amplification of LSU rDNA were D1R (Scholin *et al*. 1994) and 28–1483R (Daugbjerg *et al*. 2000), and internal primers for cycle sequencing including those originally arranged are given in Table 1. The polymerase chain reaction (PCR) was carried out through thermal conditions of an initial denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 3 min. The reaction was completed with a final elongation at 72°C for 10 min. The sufficient amplification was confirmed by 1.0% agarose gel electrophoresis, then the successfully obtained PCR product was purified using a Microcon YM-100 centrifugal filter device (Millipore, Billerica, MA, USA) to remove the remnants of primer used in previous amplifications. The cycle sequencing reaction was carried out using an ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA, USA) following the manufacturer's protocol with the internal primers given in Table 1. The gene sequences determined in the present study were deposited in the GenBank database under accession numbers AB288382 to AB288386.

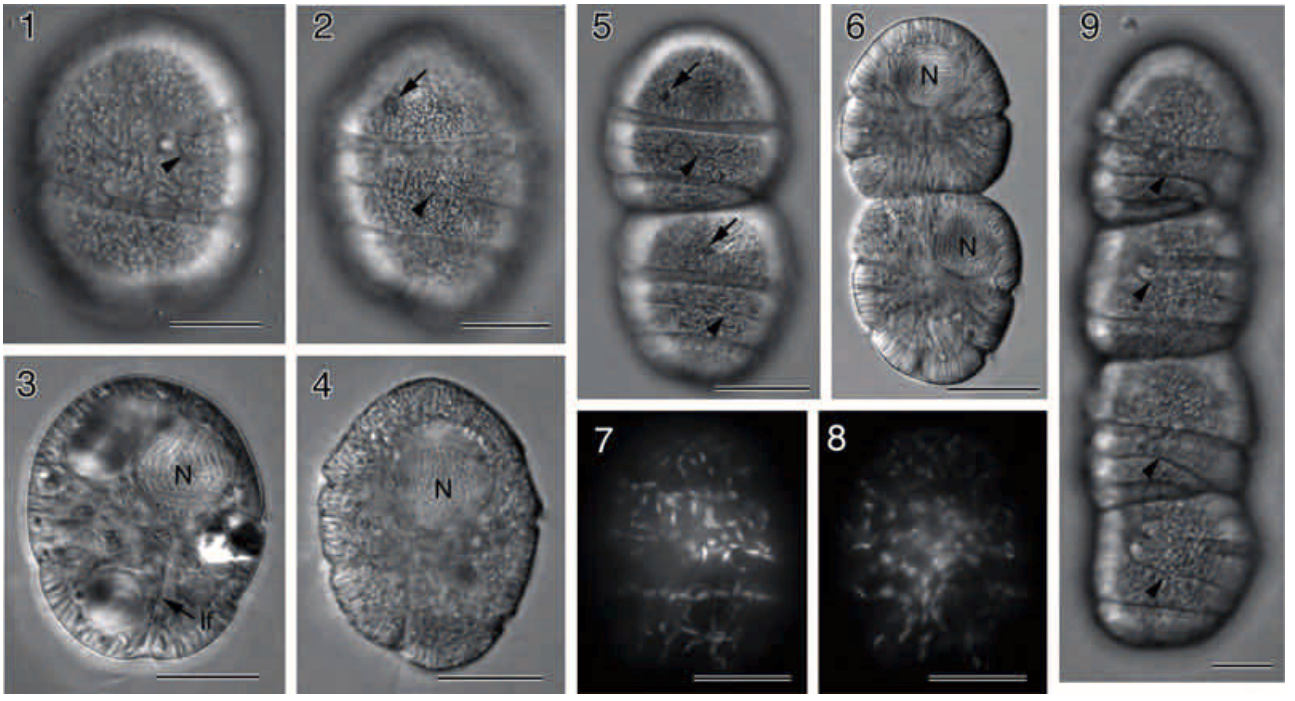
Table 1. List of oligonucleotide primers used in the present study

Code	SD	Primer sequence (5′ – 3′)	Anneals to†
D1R‡	F	ACCCGCTGAATTTAAGCATA	24–31
D2Ra‡,††	F	TGAAAAGGACTTTGAAAAGA	365–384
3R††	R	TCTTTTCAAAGTCCTTTTCA	384–365
D3A§,††	F	GACCCGTCTTGAAACACGGA	708–727
D2C‡,††	R	CCTTGGTCCGTGTTTCAAGA	733–714
6F††	F	TAGTAGCTGGTTCCCTCCGA	992–1011
D3B‡,††	R	TCGGAGGGAACCAGCTACTA	1011–992
8F††	F	TGTGTAACAACTCACCTGCC	1318–1337
28–1483R¶	R	GCTACTACCACCAAGATCTGC	1504–1483

†Annealing positions to *Prorocentrum micans* (Lenaers *et al*. 1989). ‡Referred to Scholin *et al*. (1994). §Referred to Nunn *et al*. (1996). ¶Referred to Daugbjerg *et al*. (2000). ††Internal primers used for cycle sequencing. F, forward; R, reverse; SD, synthesis direction.

Large subunit rDNA sequences of *Cochlodinium* determined in this study were aligned with those of selected dinoflagellates (GenBank accession numbers are given in Fig. 22) using the Clustal X 1.8 computer algorithm, and were subsequently edited manually at the part that was obviously misaligned. For neighbor-joining (NJ) and maximum likelihood (ML) analyses, the general time reversible (GTR) model with among-sites rate variation approximated by a discrete gamma distribution (G) with four rate categories plus the proportion of invariant sites (I) was selected by ModelTest 3.04 (Posada & Crandall 1998). Phylogenetic trees were constructed using PAUP* 4.0b10 (Swofford 2003). Bootstrap support (BS) values (Felsenstein 1985) were estimated based on the method of gamma-weighted NJ (NJ, 1000 replicates), maximum parsimony (MP, 1000 replicates) and ML (100 replicates).

The SSU rDNA sequences of *C. fulvescens* cultures collected from off Tachibana Bay, Japan and Hurun Bay, Indonesia were determined by the same protocols mentioned above. Primer sets used for SSU rDNA were identical to those used in Matsuoka *et al*. (2006). These SSU rDNA sequences of *C. fulvescens* are available under GenBank accession numbers AB288381 and AB288380.



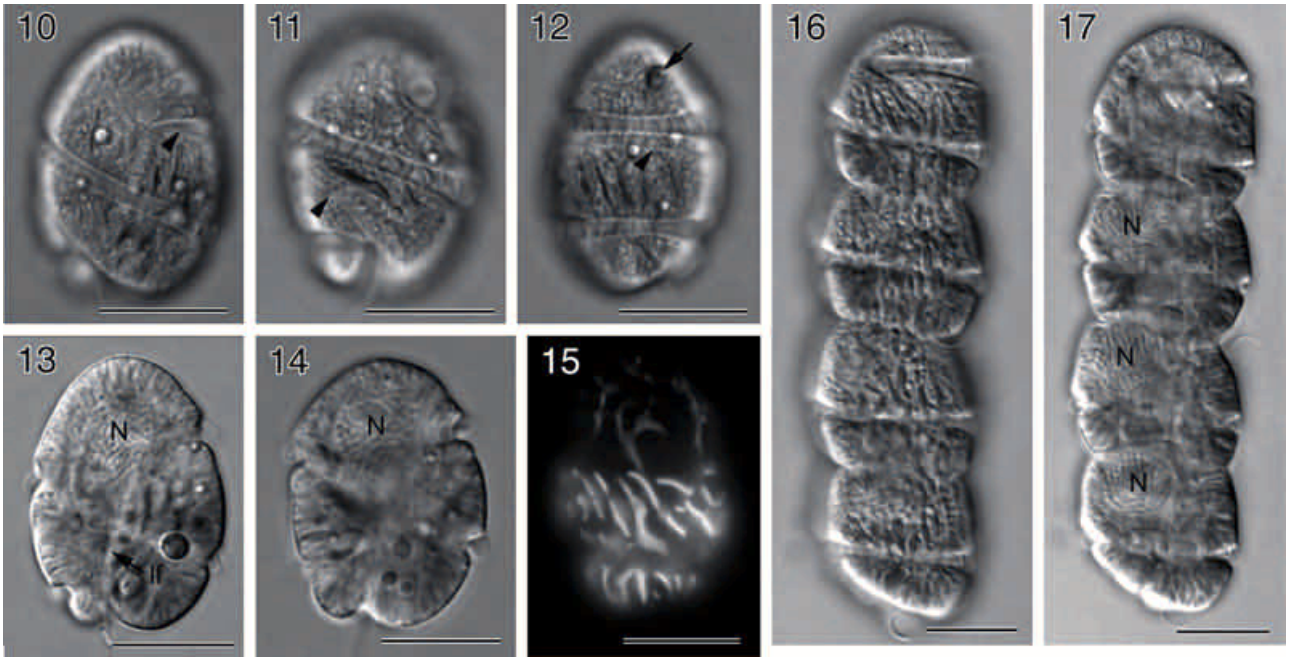
Figs 1–9. Light and fluorescence micrographs of *Cochlodinium fulvescens* Iwataki, Kawami et Matsuoka sp. nov. 1. Ventral view showing the starting position of the narrow sulcus (arrowhead). 2. Dorsal view showing an orange pigmented body (arrow) located in the epicone, narrow sulcus (arrowhead) running in the intermediate position of the cingulum. 3,4. Optical cross section, a spherical dinokaryotic nucleus (N) is positioned in the anterior part of the cell. The longitudinal flagellum (lf) runs in the cell. 5. Dorsal surface of two cells forming a chain, showing the positions of an orange pigmented body (arrows) and sulcus (arrowheads). 6. Deeper part from the surface of the same cells of Figure 5, nucleus (N) located in the anterior. 7. Autofluorescence of chloroplast, surface view indicates granular chloroplasts positioned especially along the cingulum and sulcus. 8. Same cell shown in Figure 7 showing chloroplasts also positioned in the center of the cell. 9. Cell chain consisting of four cells, surface view. Note the positions of sulcus (arrowheads). Scale bars = 20 μm.

RESULTS

Morphology of *Cochlodinium fulvescens*

Solitary cells of *Cochlodinium fulvescens* are subspherical to ellipsoidal, range 37.5–57.5 μm (mean = 45.8 μm, $n = 30$) in length and 30.0–42.5 μm (mean = 35.3 μm, $n = 30$) in width (Figs 1–4,18,19). The epicone was hemispherical, and the hypocone with the posterior end slightly excavated by the sulcal notch (Figs 3,4). The cingulum surrounded the cell approximately twice, harboring the transverse flagellum (Figs 1,2). The sulcus was diverged from the starting position of the cingulum and encircling the cell about once (Figs 1,2,5,9), but it was rather narrow and never held the longitudinal flagellum in this structure. The longitudinal flagellum went straight towards the antapical end, through the cell (Fig. 3). On the dorsal side, the sulcus ran at an approximately intermediate position between the upper and lower part of the cingulum (Figs 2,5). A spherical dinokaryotic nucleus was located in the anterior part of the cell (Figs 3,4). An eyespot-like amorphous reddish orange-pigmented body was located in the dorsal side of the epicone (Figs 2,5). Many translucent and fusiform trichocysts were densely present in the periphery of the cell (Figs 3,6). Chloroplasts radiated from the center of the cell (not shown) and were located also in the periphery (Figs 7,8). The peripheral chloroplasts are usually granular and brownish in culture conditions, and are distributed along the rim of the cingulum and sulcus (Fig. 7). Since they were rather small, the cell itself was apparently pale yellow. Although not visible in the photos, a shallow groove similar to the apical groove, originating at the starting position of the cingulum that elongates to the anterior and curves along the cingulum, was observed under light microscopy. This species forms a chain consisting of two to four cells (Figs 5,6,9). Cells composed of the chain were closely contacting and each boundary was almost flat (Fig. 6), therefore the intermediate cells in the chain were longitudinally compressed (Fig. 9).

Two- or four-cell chains were observed in field samples of *C. fulvescens*, while solitary or two-cell chains mainly occurred in cultures established by isolating a cell chain. Resting cysts or hypnozygotes were not found in all cultures.



Figs 10–17. Light and fluorescence micrographs of *Cochlodinium polykrikoides*. 10. Ventral view of a single cell showing the torsion of the cingulum with approximately two turns. The sulcus (arrowhead) originates from the starting position of the cingulum. 11. Ventral surface, note the position of sulcus (arrowhead). 12. Dorsal surface, an orange pigmented body (arrow) situated in the epicone. 13,14. Optical cross section showing the longitudinal flagellum (lf) originates at the beneath the cingulum and extends downward through the cell. A nucleus (N) located in the anterior part of the cell. 15. Autofluorescence of chloroplasts, surface view shows rod-shaped chloroplasts obliquely extends near the longitudinal axis, which distributes specifically beneath the cingulum. 16. Surface of cell-chain consisting of four cells. 17. Deeper part near the nucleus (N) of the four cell-chain. Scale bars = 20 μm.

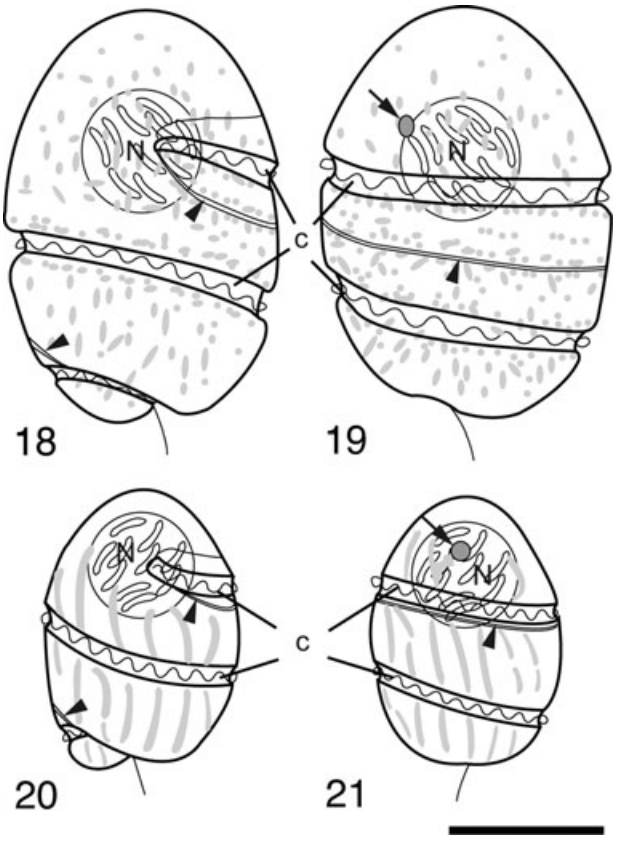
Morphology of *Cochlodinium polykrikoides*

Single cells of *Cochlodinium polykrikoides* were ellipsoid, 28.0–40.0 μm (mean = 33.3 μm, $n = 30$) in length and 20.0–28.0 μm (mean = 24.1 μm, $n = 30$) in width (Figs 10–14,20,21). The cingulum surrounded the cell approximately twice (Figs 10–12). The sulcus was rather narrow and located just below the cingulum on the dorsal side (Figs 10–12). The longitudinal flagellum was never harbored in the narrow sulcus, and originated beneath the starting position of the cingulum and inside the cell (Fig. 13). A spherical dinokaryotic nucleus was located in the anterior part of the cell (Figs 13,14) and an orange-pigmented body was positioned in the dorsal side of the epicone (Fig. 12). Chloroplasts were rod-shaped, extending slightly oblique from a longitudinal axis in healthy cells, and were densely distributed peripherally and brownish in color (Fig. 15). A shallow groove originating at the starting position of the cingulum, elongating to the anterior and curving along the cingulum, and the other structure similar to a horseshoe-shaped apical groove at the apex of the cell could be faintly observed (Fig. 10). The more detailed structure remained obscure. Cells usually formed chains consisting of more than four, sometimes 16 cells (Figs 16,17). Since the anterior part of the cell somewhat extended into the posterior part of the upper cell in the cell-chain, the borders of cells were slightly curved (Fig. 17).

LSU rDNA and SSU rDNA sequence analyses

One partial LSU rDNA sequence (1440 bp) was decided from a culture of *C. fulvescens* collected from Tachibana Bay. Four LSU rDNA sequences (*c.* 1500 bp) of *C. polykrikoides* cultures from Japanese coastal waters were also determined, and appeared to be identical to one another. Between LSU rDNA of *C. fulvescens* and *C. polykrikoides*, 14.9% sequence divergences were found in the D1–D6 region. After removing a hyper variable site D2, 11.0% sequence divergence was found. The sequence divergences between *C. fulvescens* and other selected unarmored dinoflagellates were larger than that between *C. fulvescens* and *C. polykrikoides* (>18.6% in D1–D6, and >11.3% in D1–D6 without D2).

Three methods for phylogenetic tree construction (NJ, MP and ML) were attempted, but differences were



Figs 18–21. Diagrammatic drawings of *Cochlodinium fulvescens* and *C. polykrikoides*. 18. Ventral view of *C. fulvescens*. 19. Dorsal view of *C. fulvescens*. 20. Ventral view of *C. polykrikoides*. 21. Dorsal view of *C. polykrikoides*. Arrow, orange pigmented body; arrowheads, sulcus; c, cingulum; N, nucleus. Scale bar = 20 μm.

not detected among these topologies as to the phylogenetic relationship of *Cochlodinium* species analyzed in this study. A gamma-weighted ML tree with BS values based on those three methods is given in Figure 22. In the phylogenetic tree based on LSU rDNA sequences (D1–D6 without D2), dinoflagellates formed a well-supported clade (NJ/MP/ML = 100%/100%/100%), but relationships among dinoflagellate taxa were obscure. *Cochlodinium fulvescens* was closely related to *C. polykrikoides* as a sister group and this relationship was supported by high BS values (94%/97%/99%). The related genera to this *Cochlodinium* clade were shown to be *Gymnodinium* and *Togula* in the ML tree (Fig. 22), but these relationships were not supported by bootstrap analyses.

Two SSU rDNA sequences of *C. fulvescens* collected from off Tachibana Bay, Japan and Hurun Bay, Indonesia were identical. The two *C. fulvescens* sequences possess 9.61% (167/1738 bp) of sequence divergence to those of *C. polykrikoides* of Korean strains (AY421781, AY421782, AY347309, AY421779 and AY421780).

Diagnoses

Cochlodinium fulvescens Iwataki, Kawami et Matsuoka sp. nov. (Figs 1–9)

Dinoflagellata athecata photosynthetica, solitaria aut conformatio 2–4 cellulae concatenatum cum 2–4 culluli. Cellulae subsphaericum vel breviter ellipsoidalium, 37.5–57.5 μm longae, 30.0–42.5 μm crassae, cum cingulo circum fere bis et granulo rufescentis in parte anterio-dorsali. Haec species a *Cochlodinium polykrikoides* Margalef differt et magnitudine cellula grandis, chloroplasto granularis, sulco loco intermediae cinguli.

A photosynthetic unarmored dinoflagellate, solitary or forming cell chains. Cells subspherical to shortly ellipsoidal, 37.5–57.5 μm long, 30.0–42.5 μm wide, with the cingulum encircling the cell about twice, an orange pigmented body located in the dorsal side of the epicone. This species differs from *C. polykrikoides* by larger cell size, granular chloroplasts and the sulcus located in the intermediate position of the cingulum.

Holotype: Figure 9.

Type locality: off Tachibana Bay, Nagasaki, Japan (32°32′N, 129°42′E).

Etymology: The Latin word *fulvescens* is flavescent in English, named for the color of the cell. This species appears to be pale yellow compared to *C. polykrikoides*.

DISCUSSION

An unarmored dinoflagellate *Cochlodinium fulvescens* is photosynthetic with a cingulum surrounding the cell approximately twice, and forming cell chains consisting of four cells. These morphological features, especially the cingulum, clearly differ from other unarmored, photosynthetic, chain-forming dinoflagellates with the cingulum surrounding the cell only once – *Gymnodinium catenatum* Graham and *Gymnodinium impudicum* (Fraga et Bravo) G. Hansen et Moestrup, and a pseudocolony-forming photosynthetic species *Pheopolykrikos hartmannii* (Zimmermann) Matsuoka and Fukuyo. The LSU rDNA phylogenies also suggested the distant relationship between the above three species and *C. fulvescens*. Based on the characteristics of the cingulum, this species is clearly assignable to the genus *Cochlodinium* according to the generic criterion proposed by Schütt (1896).

In the genus *Cochlodinium*, more than 40 species have been described (Kofoid & Swezy 1921; Lebour 1925; Schiller 1933); however, most species previously reported are heterotrophic. Only four species in the genus have been described to possess chloroplasts and form cell chains: *C. catenatum* Okamura, *C. geminatum* (Schütt) Schütt, *C. heterolobatum* Silva and *C. polykrikoides* (Schütt 1895, 1896; Okamura 1916; Margalef 1961; Silva 1967). The cell of *C. geminatum*

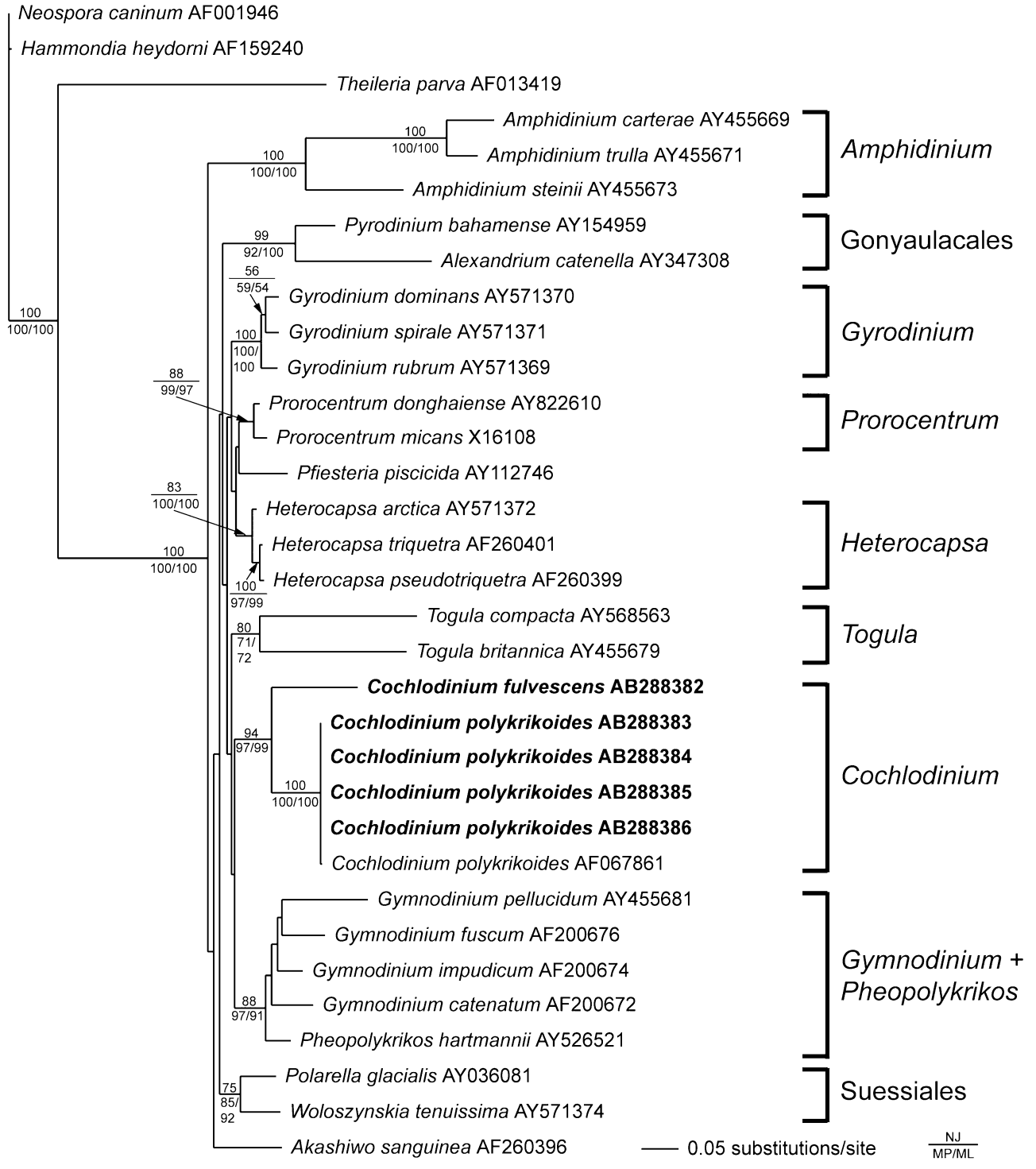


Fig. 22. Phylogenetic tree inferred from the gamma-weighted maximum likelihood (ML) analysis using PAUP* ver. 4.0b10, based on partial large subunit ribosomal DNA (LSU rDNA) sequences (D1–D6 without D2, 1315 bp in *Cochlodinium fulvescens*). Model parameters estimated from the data by ModelTest. Bootstrap support (BS) values above 50% are shown; BS values of the neighbor-joining (NJ) analysis are given above, while those of the maximum parsimony (MP) and ML are lower left and lower right, respectively. Accession numbers are indicated after species names in each taxon. Bold type indicates *Cochlodinium* species analyzed in this study.

(as *Gymnodinium geminatum* Schütt) has the epicone and hypocone different in shape and the nucleus located in the center of the cell, and the broadest part of the cell is at the rim of the cingulum in the original figure (Schütt 1895). These characteristics are never observed in *C. fulvescens*. On the other hand, *C. polykrikoides* shares many morphological features with *C. fulvescens*, namely, the narrow sulcus encircling the cell approximately once, the presence of an orange-pigmented body (stigma) at the dorsal side, and spherical nucleus located in the anterior part of the cell. Our phylogenetic tree also suggests the sisterhood between *C. polykrikoides* and *C. fulvescens*. Compared to *C. polykrikoides*, however, *C. fulvescens* is somewhat larger in size and rather rounded in shape, and have reticulate to granular chloroplasts, not a rod-like appearance. The most distinctive feature of *C. fulvescens* discernible from *C. polykrikoides* is the position of the narrow sulcus, which is diverged below the starting point of the cingulum and readily divagates, and runs the median of upper and lower parts of the cingulum, whereas the sulcus of *C. polykrikoides* lies just beneath the cingulum at least on the dorsal side. Based on these morphological differences, *C. fulvescens* can be evidently distinguished from *C. polykrikoides*. Cells of *C. fulvescens* appear to be wider and pale yellow compared to those of *C. polykrikoides* in the field sample. The other two chain-forming photosynthetic species, *C. catenatum* and *C. heterolobatum*, have no individual features from *C. polykrikoides*, and the similarities to *C. polykrikoides* have frequently been discussed (Matsuoka & Iwataki 2004). According to original descriptions, *C. catenatum* has rod-like chloroplasts extending longitudinally, and *C. heterolobatum* has the sulcus located just below the cingulum (Okamura 1916; Silva 1967). This morphological evidence strongly indicates their affinity to *C. polykrikoides*, not to *C. fulvescens*.

Several catenate and photosynthetic *Cochlodinium* species similar to *C. polykrikoides* have so far been reported (Yuki & Yoshimatsu 1989; Whyte *et al*. 2001). Among these undescribed *Cochlodinium* species, *Cochlodinium* sp., which Yuki & Yoshimatsu isolated from the Seto Inland Sea in Japan in 1985, has a strong morphological resemblance to *C. fulvescens*. The cell of *Cochlodinium* sp. of Yuki and Yoshimatsu is ellipsoid with the cingulum of about two turns, a stigma situated at the dorsal side, and the size range (38–50 μm) is also very similar to *C. fulvescens*. According to Yuki and Yoshimatsu (1989), interestingly, this *Cochlodinium* sp. formed a red tide and showed the harmful effect to juvenile fishes indicated more lethal feature than *C. polykrikoides*. Since the cell of *C. fulvescens* tends to be broadening and forming shorter cell chains compared to *C. polykrikoides*, *Cochlodinium* sp. reported by Whyte *et al*. (2001) off Vancouver Island, Canada, morphologically resembles *C. fulvescens*. This *Cochlodinium* sp. forms a pairs of cells in common according to them, and chloroplasts do not extend longitudinally. However, the most decisive characteristic of *C. fulvescens*, the position of sulcus, is not confirmed in their report.

In the phylogenetic trees based on the partial LSU rDNA sequences, *C. fulvescens* was positioned at the sister group of *C. polykrikoides* with a high BS value. These two *Cochlodinium* species has several common morphological features that have never been found in other photosynthetic *Cochlodinium* species, such as: (i) a narrow sulcus encircles the cell approximately once and does not harbor the longitudinal flagellum; (ii) the spherical nucleus is located in the epicone; and (iii) an orange-pigmented body is situated in the anterior part of the dorsal side. These features are consistent with the close phylogenetic affinity between *C. fulvescens* and *C. polykrikoides*. Specifically, the orange-pigmented body (stigma) located in the dorsal side of the epicone is a unique characteristic to these two *Cochlodinium*, which has never been found in other dinoflagellates previously described. Our phylogenetic analysis failed to identify the dinoflagellate group closely related to the chain-forming *Cochlodinium* clade due to a lack of phylogenetic resolution.

Recent phylogenetic studies have revealed the polyphyly of unarmored dinoflagellate genera *Amphidinium*, *Gymnodinium* and *Gyrodinium*, which had been distinguished by characteristics of the cingulum (Daugbjerg *et al*. 2000; Flø Jørgensen *et al*. 2004; Takano & Horiguchi 2004). Regarding the genus *Cochlodinium*, no taxonomic study based on molecular phylogeny has been attempted because of their scarce occurrence and difficulty in establishing laboratory cultures. Considering the fact that the type species of the genus *Cochlodinium*, *C. strangulatum* (Schütt) Schütt, is a heterotrophic species with tenuous cell surface striations and circle-shaped apical grooves (Schütt 1896; Kofoid & Swezy 1921; Takayama 1998), this unarmored genus characterized by the feature of cingulum is probably polyphyletic, as well as other unarmored genera. We tried to examine the apical groove structure of *C. fulvescens*, but unfortunately any procedure that we attempted for scanning electron microscopy (SEM) preparation (e.g. Takayama 1998) could not reveal the structure due to its fragility. Nevertheless, the affiliation of this new species should follow the traditional classification system until the phylogenetic relationship in the genus *Cochlodinium* is disclosed.

Cochlodinium polykrikoides is one of the serious harmful red tide forming species, but the harmful effect for fish species has not been fully clarified for *C. fulvescens* yet. Currently, it is unclear whether *C. fulvescens* is involved in harmful algal blooming events, but investigations on harmful *Cochlodinium* should be carried

out by distinguishing these two similar species so that we can understand the nature of their distribution and behavior.

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